Current Biology *16*, 680–686, April 4, 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.03.024

Report

Loss of Glial Lazarillo, a Homolog of Apolipoprotein D, Reduces Lifespan and Stress Resistance in *Drosophila*

Diego Sanchez,[1,3,4] Begoña López-Arias,[2] Laura Torroja,[2] Inmaculada Canal,[2] Xiaohui Wang,[3] Michael J. Bastiani,[3] and Maria D. Ganfornina[1,3,4,*]

[1] Departamento de Bioquímica y Fisiología y Genética Molecular-IBGM
Universidad de Valladolid-CSIC
47003 Valladolid
Spain

[2] Departamento de Biología
Universidad Autónoma de Madrid
28049 Madrid
Spain

[3] Biology Department
University of Utah
Salt Lake City, Utah 84112

Summary

The vertebrate Apolipoprotein D (ApoD) is a lipocalin secreted from subsets of neurons and glia during neural development and aging [1–3]. A strong correlation exists between ApoD overexpression and numerous nervous system pathologies as well as obesity, diabetes, and many forms of cancer [4, 5]. However, the exact relationship between the function of ApoD and the pathophysiology of these diseases is still unknown. We have generated loss-of-function *Drosophila* mutants for the *Glial Lazarillo* (*GLaz*) gene [6], a homolog of ApoD in the fruit fly, mainly expressed in subsets of adult glial cells. The absence of *GLaz* reduces the organism's resistance to oxidative stress and starvation and shortens male lifespan. The mutant flies exhibit a smaller body mass due to a lower amount of neutral lipids stored in the fat body. Apoptotic neural cell death increases in aged flies or upon paraquat treatment, which also impairs neural function as assessed by behavioral tests. The higher sensitivity to oxidative stress and starvation and the reduced fat storage revert to control levels when a GFP-*GLaz* fusion protein is expressed under the control of the *GLaz* natural promoter. Finally, *GLaz* mutants have a higher concentration of lipid peroxidation products, pointing to a lipid peroxidation protection or scavenging as the mechanism of action for this lipocalin. In agreement with Walker et al. ([7], in this issue of *Current Biology*), who analyze the effects of overexpressing *GLaz*, we conclude that *GLaz* has a protective role in stress situations and that its absence reduces lifespan and accelerates neurodegeneration.

*Correspondence: opabinia@ibgm.uva.es
[4] These authors contributed equally to this work.

Results and Discussion

Flies Lacking GLaz Are More Sensitive to Oxidative and Starvation Stress

We generated two loss-of-function alleles for *GLaz* by imprecise excision of a nearby EP element located 1425 bp upstream of the transcription initiation site (see Figure S1 in the Supplemental Data available with this article online). Flies homozygous for the two alleles, $GLaz^{\Delta 1}$ and $GLaz^{\Delta 2}$ (both null at the RNA level), are viable and fertile, suggesting that *GLaz* is not essential for embryonic or postembryonic development. Since oxidative stress is one of the common factors to the many situations in which ApoD is overexpressed, we tested the sensitivity of the null *GLaz* mutants to various stressors. *GLaz* mutant adults are in fact more sensitive to oxidative (Figures 1A and 1C) and starvation (Figure 1D) stress. These effects are seen in both males and females, but only male data will be considered here. Sensitivity to oxidative stress was still observed after placing the $GLaz^{\Delta 2}$ allele over a deficiency uncovering 22 genes around the *GLaz* locus, discarding the possibility that deleterious mutations elsewhere in the genome could contribute to the observed phenotype (Figure S2). A putative contribution of the neighboring gene *Spt1* was also rejected, since transheterozygous flies with the lethal allele $Spt1^{l(2)Sh1626}$ [8] and $GLaz^{\Delta 2}$ show survival curves similar to the control $GLaz^{\Delta 2/+}$ or $GLaz^{+/+}$ flies upon exposure to paraquat or H_2O_2 (see Figure S2 and Supplemental Data). Moreover, Spt1 expression levels show no significant changes in $GLaz^{\Delta 2}$ mutants (results not shown), as assessed by quantitative RT-PCR with the ProbeLibrary technology (Exiqon) and 18S as endogenous control. Together, these results suggest that GLaz protects the organism against damage caused by environmental stress. Transgenic flies overexpressing *GLaz* in nervous and muscular tissues have been independently generated by Walker et al. [7]. An excess of GLaz results in an increased resistance to oxidative stress and starvation, strongly supporting a protective role for GLaz.

The Absence of GLaz Reduces Lifespan

Oxidative damage to macromolecules is also one of the major causes of the aging process [9], and increased longevity is correlated with resistance to several forms of stress [10]. In accordance with these relationships, *GLaz* mutant adults have a reduced lifespan. The same results are obtained with the GLaz mutation placed in two genetic backgrounds. The survival curves of age-synchronized cohorts of male flies reared at 25ºC show a reduction in both the median and the maximum lifespan (Figures 1E and 1F), reflecting an early onset of senescence. Mirroring this phenotype, *GLaz* overexpression [7] results in an extended male longevity.

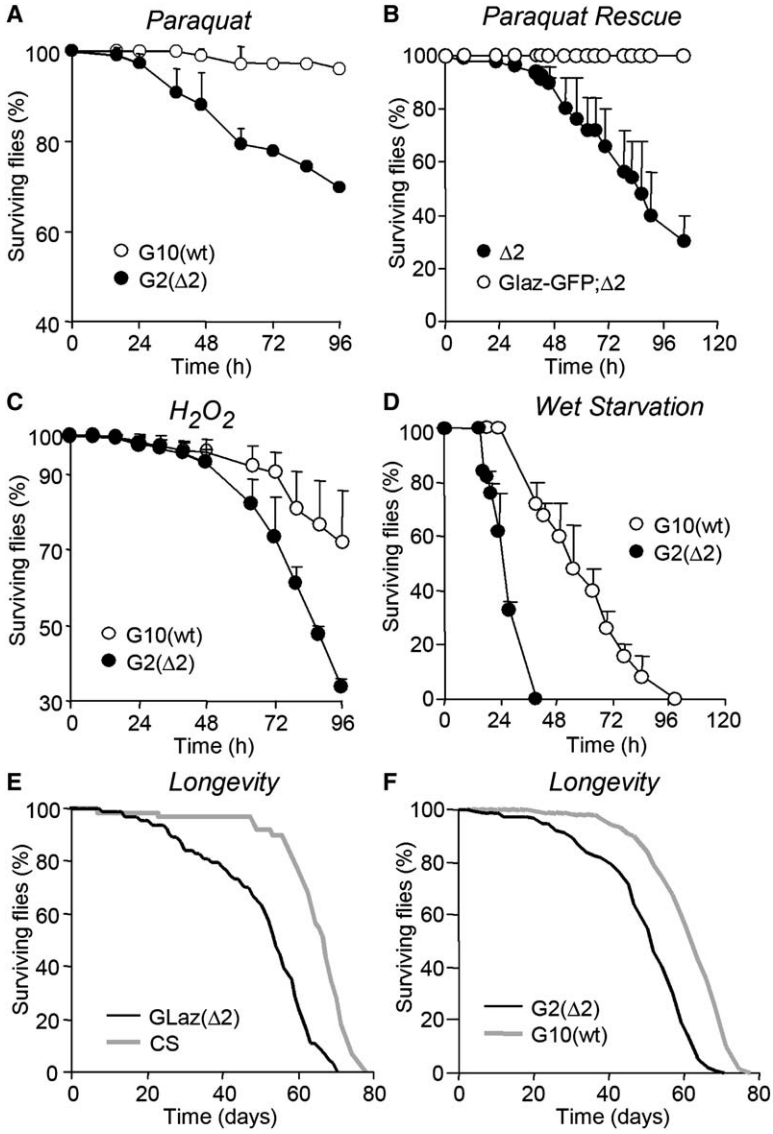

Figure 1. The Absence of *GLaz* Reduces Resistance to Oxidative Stress and Starvation and Shortens Lifespan

Sensitivity to stress is rescued if normal GLaz expression is restored.

Data in (A)–(D) are the average ± SEM from three independent experiments. Male data are shown. Log-rank test was used for statistical analysis. Flies still alive at the end of the experiment were assigned a censoring value of 1 and their survival time was considered as a minimum survival time.

(A) Survival of $GLaz^{\Delta 2/\Delta 2}$ flies compared to wild-type flies upon exposure to a ROS generator (paraquat, 20 mM) supplied in the food (n = 100/genotype); $p < 0.00001$. $GLaz^{\Delta 2/\Delta 2}$ mutants were outcrossed with the Canton-S (CS) strain. Line G2, homozygous for the $GLaz^{\Delta 2}$ allele, is compared to line G10, homozygous for the $GLaz^{+}$ allele.

(B) The sensitivity to paraquat of $GLaz^{\Delta 2/\Delta 2}$ mutant flies is reverted back to control levels when a GFP-GLaz fusion protein is expressed under the control of the *GLaz* natural promoter (n = 75/genotype); $p < 0.00001$. Paraquat was supplied in Whatman 3M filter papers soaked with 1 ml of a 10% sucrose, 20 mM paraquat solution (see Supplemental Experimental Procedures).

(C) Survival analysis upon feeding 1% H_2O_2 on day 1 and 5% H_2O_2 on subsequent days to the *GLaz* mutant and control flies (n = 150/genotype); $p < 0.00001$. H_2O_2 was supplied daily in Whatman 3M filter papers soaked with 1 ml of a 10% sucrose solution with H_2O_2.

(D) Effect of starvation on survival of $GLaz^{\Delta 2/\Delta 2}$ compared to $GLaz^{+/+}$ flies (n = 75/genotype); $p < 0.00001$. Humidity was supplied daily in Whatman 3M filter papers soaked with 1 ml of water.

(E) Lifespan determination of $GLaz^{\Delta 2/\Delta 2}$ compared to wild-type males (CS). Median survival time is reduced by 19.3% in the *GLaz* mutant (log-rank test, $p < 0.0001$, n = 65/genotype), and maximum survival time is reduced by 10.9% (Gehan's Wilcoxon test, $p = 0.0013$).

(F) Reduction of lifespan is maintained after outcrossing the $GLaz^{\Delta 2}$ mutant allele with the CS strain (see Supplemental Data) (n = 225/genotype). Median survival time is reduced by 18.1% (log-rank test: $p < 0.00001$). Maximum survival time is reduced by 12.7% (Wilcoxon test: $p < 0.0001$).

GLaz Expression Sites in the Adult Fly: The Nervous System and Hemocytes

In order to understand how a lipocalin, an extracellular lipid binding protein, can contribute to the regulation of oxidative damage in general and of aging in particular, we analyzed the expression pattern of *GLaz* in adult flies (Figure 2). We generated a reporter gene construct that drives the expression of GFP under the *GLaz* promoter (GFP-R3rd line; see Figure S1 and Supplemental Data). The expression of the reporter gene is mostly seen in the nervous system, where it is restricted to a subset of cells in the perineurial sheath surrounding brain and ganglia, some cells in the optic system (Figure 2A), and in the mushroom body (not shown). Perineurial cells surrounding the peripheral nerves and motoneuron terminal branches also show expression (Figure 2B). In addition to their stereotyped position, the lack of coexpression with neuronal markers (Figure 2A) and coincidence with expression sites of the REPO glial marker (not shown) [11] suggest that these are glial cells. This expression is consistent with both the developmental expression of *GLaz* in *Drosophila* [6] and with the main glial expression of vertebrate ApoD [1–3]. Both sequence comparisons and exon-intron structure [12, 13] already point to a close GLaz-ApoD kinship. Expression similarities suggest that regulatory regions might be conserved as well. Outside the nervous system, expression is observed only in the proventriculus (not shown) and in cells located in the hemocoel, which are probably hemocytes (Figure 2C).

We also generated a construct that drives the expression of a GLaz-GFP fusion protein under the same native

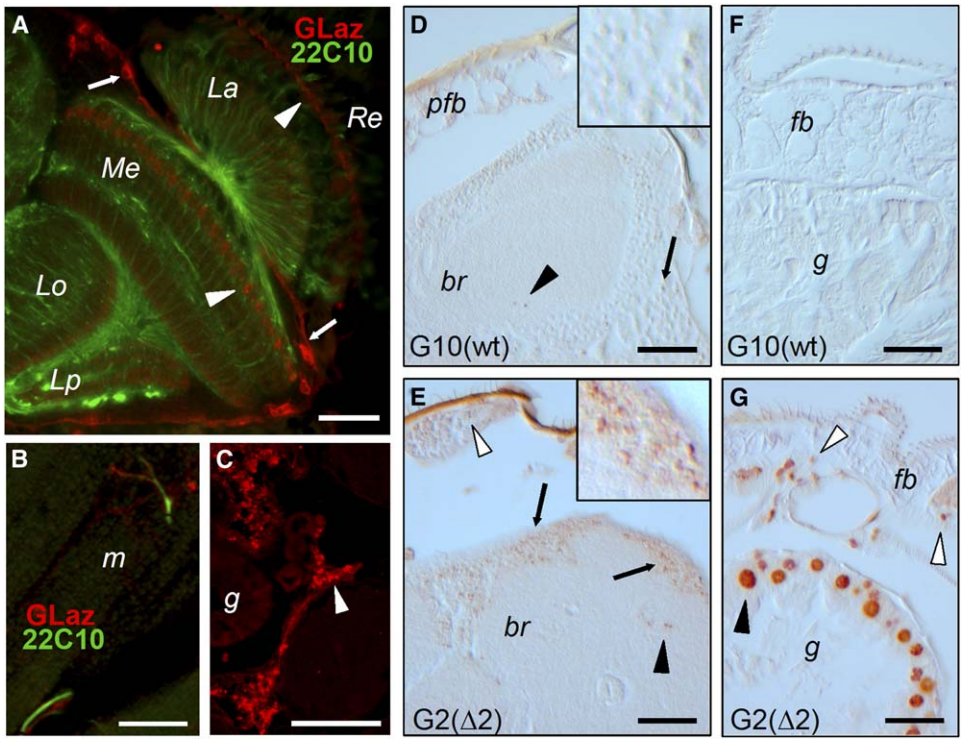

Figure 2. *GLaz* Is Expressed in Adult Glial Cells and Hemocytes

In the absence of *GLaz*, apoptotic cell death increases within and outside the nervous system.

(A–C) Expression of *GLaz* in the adult fly. GFP reporter expression was assayed by anti-GFP immunohistochemistry on horizontal paraffin sections of 3-day-old adult flies carrying the GFP reporter construct R3rd (red labeling in [A]–[C]). Monoclonal antibody 22C10 was used as a neuronal marker in (A) and (B) (green labeling). Anterior is up.

(A) In the brain, the transcriptional reporter is expressed in the perineurial sheath glia (arrows) and in subsets of cells in the medulla neuropil, the lamina (triangles), and the lobula and lobula plate in the optic lobe. The location of the *GLaz* driven GFP expression in these cells suggests its glial origin.

(B) In muscle fibers (m), peripheral nerves labeled by 22C10 are surrounded by GLaz-GFP-positive perineurial glia.

(C) *GLaz*-reported labeling in cells in the hemocoel.

(D–G) Patterns of apoptotic cell death upon loss of GLaz function in adult young flies after exposure to experimental oxidative stress. See text for quantitative analysis of TUNEL-positive cells. Pericerebral fat body (white triangle in [E]) as well as cells in the brain show apoptotic cell death in paraquat-treated *GLaz* mutants. Apoptotic cells are observed in the brain cortex (arrows in [D], [E], and inset in [E]) and in the brain neuropil (probably glial cells, black triangles in [D] and [E]).

(F and G) TUNEL-positive cells are found in the digestive tract (black triangle in [G]) and the abdominal fat body (white triangles in [G]).

Abbreviations: br, brain; fb, fat body; g, gut; La, lamina; Lo, lobula; Lp, lobula plate; m, muscle; Me, medulla; pfb, pericerebral fat body; Re, retina.

Scale bars equal 50 μm (C) or 25 μm (A, B, D–G).

GLaz promoter (see Figure S1 and Supplemental Data). The construct uses the genomic *GLaz* sequence, including introns, so all the regulatory regions within the locus are expected to be present. Two independent insertions of this construct (on the 2nd and X chromosomes) yield an expression pattern (not shown) similar to the transcriptional reporter construct. Thus, a supply of GLaz within the nervous system is provided by secretion from glial cells, while secretion from hemocytes could generate a systemic supply of GLaz.

The Effects of *GLaz* Mutation on Motor Activity Impairment Are Enhanced under Oxidative Stress Conditions

The expression pattern within the nervous system led us to evaluate whether the mutants show an age- and oxidative stress-dependent nervous system functional impairment. Locomotor behavior has been used extensively to evaluate neurodegeneration and age-related functional decline [14]. We found no differences in running, climbing, or flying abilities of young mutant and control flies (not shown). We then tested 21-day-old flies, when the mortality risk has increased only slightly for the mutant males, and we found that *GLaz* mutant males significantly reduce their climbing ability (Figure 3A). Although senescence of all motor activities tested does occur in females, no effect of genotype was observed (not shown). These data are compatible with a mild acceleration of motor activity senescence in males lacking GLaz.

After 24 hr of exposure to paraquat, *GLaz* mutant flies of both sexes perform significantly worse than controls in all tests (Figure 3B). An increased level of apoptotic death has been reported in flight and leg muscles upon treatment with oxidative stressors and during normal aging in wild-type flies [15]. This apoptotic pattern supports the notion that the normal decline of locomotor functions with age is due to accumulation of oxidative damage disturbing neuromuscular function. The enhancement of locomotor decline in *GLaz* mutants

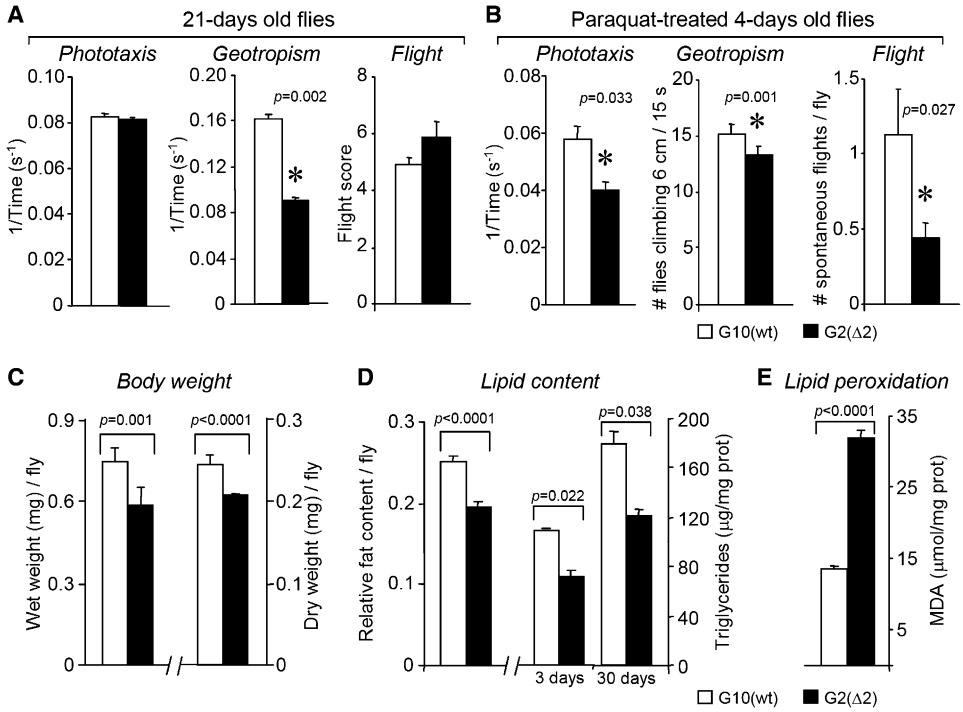

Figure 3. Loss of *GLaz* Function Leads to Functional Decline of Locomotor Activity and to Alterations in Lipid Storage and Lipid Oxidation State

Age-related (A) and oxidative stress-triggered (B) functional decline of locomotor activities. Geotropism and phototaxis responses were evaluated as described by Palladino et al. [29]. Flies collected and reared as for the longevity analyses (see Supplemental Data) were tested in groups of 10 at 3–4 days (not shown) or 20–21 days of age. Male data are shown. Scores are represented as 1/t to facilitate visual comparison with other tests results. A better performance gets a higher score in all tests.

(A) No difference between genotypes is observed in phototaxis, but $GLaz^{\Delta 2/\Delta 2}$ aged males get a lower score in geotropism since they take significantly longer to climb the same distance (n = 50/genotype). Flight performance was scored following Benzer [30]. No difference between genotypes is observed in flying ability (n = 150/genotype).

(B) After 24 hr of feeding with 20 mM paraquat supplied in filter paper, locomotor activities in the three behavioral tests were significantly impaired in $GLaz^{\Delta 2/\Delta 2}$ mutants as compared to paraquat-treated control flies. Phototaxis was evaluated as above. Methods imposing a less strenuous exercise to the flies were chosen to evaluate geotropism and flying abilities. n = 20/genotype. Female data are shown. Percent number of flies climbing over a 6 cm line in 15 s is recorded in 4 consecutive trials. Spontaneous number of flights over 4 intervals of 2 min is recorded.

(C–E) Absence of GLaz results in a smaller body mass due to a lower amount of neutral fats and in accumulation of lipid peroxidation products.

(C) Body weight differs significantly between $GLaz^{\Delta 2/\Delta 2}$ and $GLaz^{+/+}$. Mutant males weigh less than $GLaz^{+/+}$ flies (n = 200/genotype).

(D) Relative fat content in $GLaz^{\Delta 2/\Delta 2}$ flies is significantly smaller than in control flies (n = 200/genotype). Levels of triglycerides normalized to protein content also differ significantly between genotypes in young and aged flies (n = 70/genotype).

(E) Levels of free malondialdehyde (MDA), the most abundant product derived from unstable lipid peroxides, are highly increased in *GLaz* null mutants compared to control flies. Results are shown normalized to protein content. Experiments performed in triplicate (n = 150/genotype).

Data are represented as mean ± SD. Statistical differences assayed by unpaired two-sided Student's t test.

suggests that this damage is intensified when GLaz is not present. However, can the observed effects be causally linked to a nervous system defect or to a more widespread damage affecting other tissues?

Apoptotic Cell Death Increases within and outside the Nervous System in *GLaz* Mutants upon Aging or under Oxidative Stress Treatment

In agreement with Zheng et al. [15], we detect low signs of apoptosis within the nervous system of aged wild-type flies (as detected by TUNEL, 8 ± 4 cells/section), but a significant increase in apoptotic cell death is observed in the aged $GLaz^{\Delta 2/\Delta 2}$ brain (25 ± 6 TUNEL-positive cells/section, t test p = 0.003). Apoptosis in neural cells also increases when young $GLaz^{\Delta 2/\Delta 2}$ flies are exposed to paraquat (95 ± 35 TUNEL-positive cells/section; Figure 2E and inset), while levels in the wild-type nervous system remain low (33 ± 6 TUNEL-positive cells/section, t test p = 0.007; Figure 2D). These differences in patterns of apoptosis reveal that, in normal conditions, GLaz contributes to the higher tolerance to oxidative damage of the nervous system. Therefore, at least part of the decrement in locomotor performance observed in aged and paraquat-treated mutant flies can arise from a diminished nervous system function due to the death of irreplaceable cells.

Nevertheless, the nervous system is not the only tissue in which apoptosis levels rise. The number of TUNEL-positive nuclei in the pericerebral fat body of old $GLaz^{\Delta 2/\Delta 2}$ flies (10 ± 3 TUNEL-positive cells/section) increases compared to a negligible control level (3 ± 2 TUNEL-positive cells/section, p = 0.002). Upon paraquat feeding, also described by Zheng et al. [15]), apoptotic nuclei are commonly observed in the digestive system and the abdominal and pericerebral fat body (30 ± 23 versus 8 ± 4 TUNEL-positive cells/section of $GLaz^{\Delta 2/\Delta 2}$ and wild-type flies, respectively, t test p = 0.01; Figures 2F and 2G). Thus, the loss of GLaz function seems to be altering other tissues in addition to the nervous system.

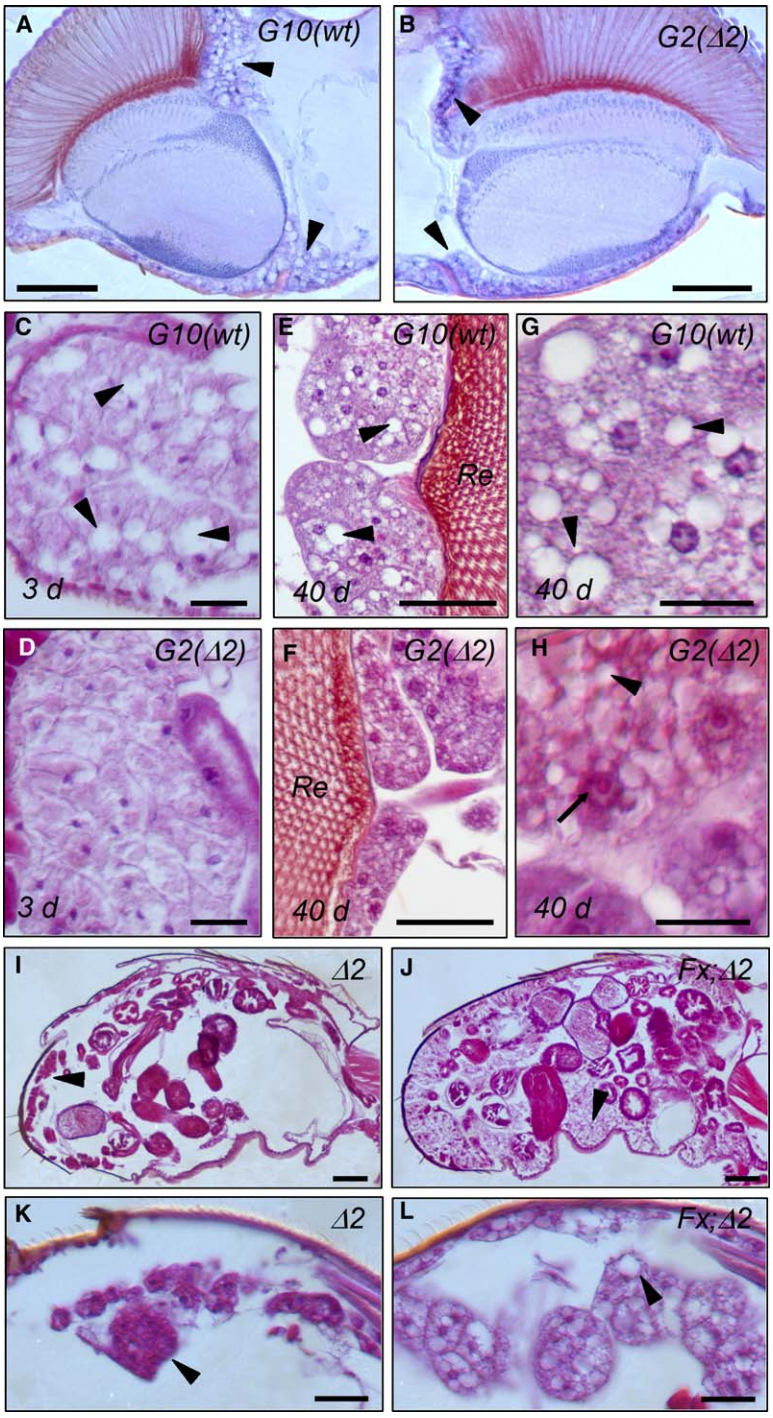

Figure 4. Lack of GLaz Profoundly Alters the Morphology of the Fat Body Tissue

The effect is reverted when a GLaz-GFP fusion protein is expressed under the control of *GLaz* natural promoter.

Standard hematoxilin-eosin histochemistry was performed on paraffin (C–L) or resin (A and B) sections. n = 15 flies/sex/genotype/condition.

(A and B) Horizontal brain sections showing the pericerebral fat body (triangles) of control and mutant 7-day-old flies. Mutant fat body tissue is markedly reduced in size.

(C and D) Abdominal fat body of 3-day-old control and $GLaz^{\Delta 2/\Delta 2}$ flies showing the reduction in size and number of lipid bodies (triangles) in mutant tissue. The same effect is observed in the pericerebral fat body (not shown).

(E–H) Aging enhances this effect as shown in the pericerebral fat body of aged control (E, G) and $GLaz^{\Delta 2/\Delta 2}$ (F, H) flies. The same effect is observed in the abdominal fat body (not shown). (G) and (H) are magnifications of (E) and (F), respectively. Arrow in (H) points to an eosinophilic cell.

(I–L) $GLaz^{\Delta 2/\Delta 2}$ fat body size reduction and decrease in lipid bodies (I, K) is restored to normal levels in mutant flies expressing the GLaz-GFP fusion protein (J, L; Fx construct) in brain and hemocytes (see Figure 2). Abdominal fat body (triangles) is shown in (I) and (J), while pericerebral fat body is shown in (K) and (L) (triangles point to lipid bodies).

Abbreviations: Re, retina. Scale bars equal 50 μm (A, B, E, F, I, J) or 20 μm (C, D, G, H, K, L).

Fat Body Physiology and Morphology Is Greatly Altered in *GLaz* Mutant Flies

Because changes in body size have also been linked to changes in longevity and stress resistance [16], we explored whether the weight of *GLaz* mutant flies differs from wild-type controls. Both sexes of $GLaz^{\Delta 2/\Delta 2}$ flies show significantly lower wet and dry body weight (Figure 3C; male data are shown).

Protein content does not differ between genotypes (not shown, t test p = 0.23). Therefore, most of the body weight variations observed can be accounted for by a marked reduction in relative fat content of $GLaz^{\Delta 2/\Delta 2}$ flies (Figure 3D; male data are shown). Further analyses show that the relative content of triglycerides (Figure 3D) in both young and old adults is significantly decreased when GLaz is absent. These biochemical parameters correlate well with the observed morphology of the fat body: both pericerebral and abdominal fat body tissue of *GLaz* mutants are markedly reduced in size (Figures 4A, 4B, and 4I). Young adult $GLaz^{\Delta 2/\Delta 2}$ flies of both sexes show fewer and smaller lipid bodies (Figures 4C and 4D), and aging worsens this effect with a reduction in the fat body mass (Figures 4E and 4F). Moreover, the limited lipid bodies present in old $GLaz^{\Delta 2/\Delta 2}$ flies are

accompanied by increased eosinophilia due to protein aggregates, a morphological indicator of forthcoming cell death (Figure 4H).

These data suggest that the absence of GLaz provokes (1) a profound reorganization in the lipid storage/lipid metabolism balance, (2) an acceleration of age-dependent neural cell death in the CNS, and (3) intense degeneration in both fat and neural tissues upon experimental oxidative stress.

Restoring *GLaz* Expression under Its Native Promoter Returns Oxidative Stress Sensitivity and Fat Body Physiology Back to Control Levels

When a GLaz-GFP fusion protein construct (GLaz-Fx line, see Figure S1 and Supplemental Data) is placed in a $GLaz^{\Delta 2/\Delta 2}$ background, the survival upon paraquat treatment returns to control levels (Figure 1B). Starvation sensitivity is also recovered, with a 66.2% increase in median survival time when comparing GLaz-Fx:$GLaz^{\Delta 2/\Delta 2}$ to $GLaz^{\Delta 2/\Delta 2}$ ($p < 0.00001$ log-rank test). The size and morphology of the fat body is restored as well (Figures 4I–4L, n = 15 flies/genotype). These results confirm that the observed phenotypes are due to the lack of GLaz protein, with negligible contributions, if any, from neighboring genes (see Supplemental Data for discussion).

The native regulatory regions used here to reinstall GLaz expression in the null mutants seem to control the level of expression within physiological levels. Interestingly, as described in the accompanying paper [7], strong overexpression in the nervous system does not provide beneficial effects on longevity. The amount of secreted GLaz might therefore be a critical factor.

We can conclude from our data that GLaz expression in a subset of cells within the nervous system and in hemocytes is sufficient to fulfill the protective function of GLaz against oxidative damage, starvation resistance, and its influence on fat body physiology (regulating lipid stocks). This also suggests that the effects seen in the fat body are exerted nonautonomously. Whether a systemic supply of GLaz provided by hemocytes is sufficient for these effects, or whether the GLaz protein produced within the nervous system has direct or indirect influences on lipid management by fat body cells can not be discerned. However, it is interesting that brain-fat body regulatory neurohormonal circuits are proposed to control nervous system and somatic senescence, which in turn are linked to the response to nutrient levels and environmental stressors (see [17] for a review).

Lipid Peroxidation Protection or Scavenging as the Mechanism of Action for the Lipocalin GLaz

With a few exceptions, lipocalin function has been related to the binding of hydrophobic ligands. This biochemical property is put to work in different cellular and organismal contexts, generating a panoply of physiological roles, such as lipid transport [18], regulation of the innate immune system [19], regulation of cell proliferation and apoptosis [20], antiinflammatory and antibacterial function [21], as well as scavenging of toxic molecules, like heme [22] or lipid peroxidation products [23]. In addition, some lipocalins have enzymatic activities, including antioxidant activities: alfa-1-microglobulin is able to protect tissues from prooxidant molecules, inhibit oxidation, and remove oxidation products [22, 24].

The data presented so far strongly suggest that GLaz is part of a defense system acting in situations of unbalanced oxidative stress, provoked exogenously from environmental conditions or endogenously by aging or pathological situations. GLaz could act as a general scavenger of toxic products (most probably peroxidated lipids) and/or exert a protective role by preventing oxidation of lipids, which is known to occur in the CNS and fat body of aged and paraquat-treated flies [25].

This hypothesis predicts that the levels of peroxidated lipids should be higher in the *GLaz* mutants. That is in fact the case: the concentration of free malondialdehyde (MDA), the main product derived from unstable lipid peroxides, is more than two times higher in $GLaz^{\Delta 2/\Delta 2}$ flies than in control flies (Figure 3E). The levels of MDA therefore represent a measure of the degree of oxidative damage in the tissue.

How Can a Molecule Protecting against Oxidative Stress Modify Lipid Stocks in the Fat Body?

If GLaz function is exerted by lipid peroxidation protection or scavenging, then the absence of GLaz would mimic a stress condition. This would result in an accelerated senescence and neurodegeneration. Fat body cells also show more cell death upon aging in *GLaz* null mutants. However, the depletion of lipid bodies is a more generalized phenomenon within this tissue, and the lower resistance to starvation could be directly linked to this effect. Possibilities to be considered are that GLaz might exert a direct signaling function on fat body cells, or that it might perform a transport function that controls the supply of lipids in a rate-limiting fashion. However, a direct interaction with the fat body is not a requirement, since the stress provoked by the absence of a protective agent (GLaz) would secondarily become a feedback loop reverberating on the stress response signaling networks. In this scenario, depletion of lipids from the fat body in *GLaz* null mutant flies would be the result of the metabolic activation triggered, for example, by biogenic amines (dopamine and octopamine) in response to the unfavorable conditions [26]. Interestingly, ApoD has been identified as a direct target of the liver X receptors in adipose tissue [27], pointing to a role of this lipocalin in the regulation of lipid homeostasis in vertebrate fat tissue as well.

Functional Significance of GLaz/ApoD Function in Disease and Aging

In recent years, data coming from different model organisms (*C. elegans*, *Drosophila*, and mouse) (see [17] for a review) suggest that the general scaffold of the neurohormonal and signaling pathways controlling stress responses and aging has been evolutionarily conserved. The loss-of-function (this work) and the gain-of-function [7] analyses of *GLaz* in *Drosophila* clearly suggest that this lipocalin has a protective function both during experimental (mimicking disease) or endogenous (aging) oxidative stress. Although cooptions to new functions and multifunctionality [28] can not be ruled out, the GLaz function is most likely conserved in ApoD, the vertebrate lipocalin closer to the insect-vertebrate node in the gene family tree.

Evidence pointing to a role for ApoD in aging and disease has accumulated over the years. The results

presented here suggest that GLaz/ApoD is an active, nondispensable element that is predicted to influence the time course and the severity of degenerative diseases and cancer as well.

Since the *Drosophila* adult is essentially a nonregenerating postmitotic organism, functional conservation with the vertebrate ApoD is expected especially in tissues that are also mainly postmitotic, e.g., the nervous system. If genetic manipulations in vertebrate model organisms confirm this prediction, the potential use of ApoD as a therapeutic agent in neurodegenerative disorders should become a research priority.

Supplemental Data

Supplemental Data include two figures and Supplemental Experimental Procedures and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/7/680/DC1/.

Acknowledgments

We thank V. Pomies for technical assistance, D. Pérez Fernández for equipment design, and S.X. Hou for kindly providing the *Spt1* mutant flies. We thank R. Ramos (PCM, Madrid Scientific Park) for the analysis of Spt1 expression by quantitative PCR. The 22C10 monoclonal antibody was from the DSHB developed under the auspices of the NICHD and maintained by The U. Iowa, Department of Biological Sciences.

The following agencies contributed to finance this work: start-up grants to D.S and M.D.G. from the "Ramón y Cajal Program," MEC, Spain; the SGFRH Foundation; NIH grant 2/R01/NS25387-10A1 to M.J.B.; and MEC grant BMC2001-0164 and grant 02/108-0 from Fundación "La Caixa" to L.T. B.L.-A. was supported by a fellowship from the UAM, Spain.

Received: December 1, 2005
Revised: March 6, 2006
Accepted: March 6, 2006
Published: April 3, 2006

References

1. Navarro, A., Del Valle, E., and Tolivia, J. (2004). Differential expression of apolipoprotein d in human astroglial and oligodendroglial cells. J. Histochem. Cytochem. *52*, 1031–1036.
2. Sanchez, D., Ganfornina, M.D., and Martinez, S. (2002). Expression pattern of the lipocalin apolipoprotein D during mouse embryogenesis. Mech. Dev. *110*, 225–229.
3. Ganfornina, M.D., Sanchez, D., Pagano, A., Tonachini, L., Descalzi-Cancedda, F., and Martinez, S. (2005). Molecular characterization and developmental expression pattern of the chicken apolipoprotein D gene: implications for the evolution of vertebrate lipocalins. Dev. Dyn. *232*, 191–199.
4. Rassart, E., Bedirian, A., Do Carmo, S., Guinard, O., Sirois, J., Terrisse, L., and Milne, R. (2000). Apolipoprotein D. Biochim. Biophys. Acta *1482*, 185–198.
5. Sreekumar, R., Halvatsiotis, P., Schimke, J.C., and Nair, K.S. (2002). Gene expression profile in skeletal muscle of type 2 diabetes and the effect of insulin treatment. Diabetes *51*, 1913–1920.
6. Sanchez, D., Ganfornina, M.D., Torres-Schumann, S., Speese, S.D., Lora, J.M., and Bastiani, M.J. (2000). Characterization of two novel lipocalins expressed in the *Drosophila* embryonic nervous system. Int. J. Dev. Biol. *44*, 349–359.
7. Walker, D.W., Muffat, J., Rundel, C., and Benzer, S. (2006). Overexpression of a *Drosophila* homolog of Apolipoprotein D leads to increased stress resistance and extended lifespan. Curr. Biol. *16*, this issue, 674–679.
8. Oh, S.-W., Kingsley, T., Shin, H.-h., Zheng, Z., Chen, H.-W., Chen, X., Wang, H., Ruan, P., Moody, M., and Hou, S.X. (2003). A P-element insertion screen identified mutations in 455 novel essential genes in *Drosophila*. Genetics *163*, 195–201.
9. Balaban, R.S., Nemoto, S., and Finkel, T. (2005). Mitochondria, oxidants, and aging. Cell *120*, 483–495.
10. Longo, V.D., and Fabrizio, P. (2002). Regulation of longevity and stress resistance: a molecular strategy conserved from yeast to humans? Cell. Mol. Life Sci. *59*, 903–908.
11. Tix, S., Eule, E., Fischbach, K.F., and Benzer, S. (1997). Glia in the chiasms and medulla of the *Drosophila melanogaster* optic lobes. Cell Tissue Res. *289*, 397–409.
12. Sanchez, D., Ganfornina, M.D., Gutierrez, G., and Marin, A. (2003). Exon-intron structure and evolution of the Lipocalin gene family. Mol. Biol. Evol. *20*, 775–783.
13. Ganfornina, M.D., Gutierrez, G., Bastiani, M., and Sanchez, D. (2000). A phylogenetic analysis of the lipocalin protein family. Mol. Biol. Evol. *17*, 114–126.
14. Grotewiel, M.S., Martin, I., Bhandari, P., and Cook-Wiens, E. (2005). Functional senescence in *Drosophila melanogaster*. Ageing Res. Rev. *4*, 372–397.
15. Zheng, J., Edelman, S.W., Tharmarajah, G., Walker, D.W., Pletcher, S.D., and Seroude, L. (2005). Differential patterns of apoptosis in response to aging in *Drosophila*. Proc. Natl. Acad. Sci. USA *102*, 12083–12088.
16. Khazaeli, A.A., Van Voorhies, W., and Curtsinger, J.W. (2005). The relationship between life span and adult body size is highly strain-specific in *Drosophila* melanogaster. Exp. Gerontol. *40*, 377–385.
17. Tatar, M., Bartke, A., and Antebi, A. (2003). The endocrine regulation of aging by insulin-like signals. Science *299*, 1346–1351.
18. Tegoni, M., Pelosi, P., Vincent, F., Spiinelli, S., Campanacci, V., Grolli, S., Ramoni, R., and Cambillau, C. (2000). Mammalian odorant binding proteins. Biochim. Biophys. Acta *1482*, 229–240.
19. Flo, T.H., Smith, K.D., Sato, S., Rodriguez, D.J., Holmes, M.A., Strong, R.K., Akira, S., and Aderem, A. (2004). Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature *432*, 917–921.
20. Taniike, M., Mohri, I., Eguchi, N., Beuckmann, C.T., Suzuki, K., and Urade, Y. (2002). Perineuronal oligodendrocytes protect against neuronal apoptosis through the production of lipocalin-type prostaglandin D synthase in a genetic demyelinating model. J. Neurosci. *22*, 4885–4896.
21. Goetz, D.H., Holmes, M.A., Borregaard, N., Bluhm, M.E., Raymond, K.N., and Strong, R.K. (2002). The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol. Cell *10*, 1033–1043.
22. Allhorn, M., Lundqvist, K., Schmidtchen, A., and Akerstrom, B. (2003). Heme-scavenging role of alpha1-microglobulin in chronic ulcers. J. Invest. Dermatol. *121*, 640–646.
23. Lechner, M., Wojnar, P., and Redl, B. (2001). Human tear lipocalin acts as an oxidative-stress-induced scavenger of potentially harmful lipid peroxidation products in a cell culture system. Biochem. J. *356*, 129–135.
24. Allhorn, M., Klapyta, A., and Akerstrom, B. (2005). Redox properties of the lipocalin alpha1-microglobulin: reduction of cytochrome c, hemoglobin, and free iron. Free Radic. Biol. Med. *38*, 557–567.
25. Zheng, J., Mutcherson, R., 2nd, and Helfand, S.L. (2005). Calorie restriction delays lipid oxidative damage in *Drosophila melanogaster*. Aging Cell *4*, 209–216.
26. Gruntenko, N., Chentsova, N.A., Bogomolova, E.V., Karpova, E.K., Glazko, G.V., Faddeeva, N.V., Monastirioti, M., and Rauschenbach, I.Y. (2004). The effect of mutations altering biogenic amine metabolism in *Drosophila* on viability and the response to environmental stresses. Arch. Insect Biochem. Physiol. *55*, 55–67.
27. Hummasti, S., Laffitte, B.A., Watson, M.A., Galardi, C., Chao, L.C., Ramamurthy, L., Moore, J.T., and Tontonoz, P. (2004). Liver X receptors are regulators of adipocyte gene expression but not differentiation: identification of apoD as a direct target. J. Lipid Res. *45*, 616–625.
28. Ganfornina, M.D., and Sánchez, D. (1999). Generation of evolutionary novelties by functional shift. Bioessays *21*, 432–439.
29. Palladino, M.J., Hadley, T.J., and Ganetzky, B. (2002). Temperature-sensitive paralytic mutants are enriched for those causing neurodegeneration in *Drosophila*. Genetics *161*, 1197–1208.
30. Benzer, S. (1973). Genetic dissection of behavior. Sci. Am. *229*, 24–37.